

**Title:** Biomimetic Tissue Engineered Systems for Advancing Cancer Research: NCI Strategic Workshop Report

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## **Abstract**

Advanced technologies and biomaterials developed for tissue engineering and regenerative medicine present tractable biomimetic systems with potential applications for cancer research. Recently, the National Cancer Institute convened a Strategic Workshop to explore the use of tissue biomanufacturing for development of dynamic, physiologically relevant *in vitro* and *ex vivo* biomimetic systems to study cancer biology and drug efficacy. The workshop provided a forum to identify current progress, research gaps, and necessary steps to advance the field. Opportunities discussed included development of tumor biomimetic systems with an emphasis on reproducibility and validation of new biomimetic tumor models, as described in this report.

## Introduction

Successful use of three-dimensional (3D), heterotypic *in vitro* and *ex vivo* models has been widespread in cancer research, and efforts are emerging to incorporate physiological parameters such as perfusion, mechanics, and physicochemical gradients. To assess the status of the field and challenges going forward, the National Cancer Institute's Division of Cancer Biology in collaboration with the Division of Cancer Treatment and Diagnosis and the Center for Strategic Scientific Initiatives sponsored a workshop on February 26, 2014 entitled *Biomimetic Tissue Engineered Systems for Advancing Cancer Research*. Leaders in the fields of tissue engineering and regenerative medicine, biomedical engineering, cancer research, cell and molecular biology, and pharmacology convened to discuss how biomimetic technologies can play a pivotal role in advancing our understanding of cancer. Although not a comprehensive assessment, this report summarizes the findings presented in the workshop and discussions of future opportunities.

## Presentation Summaries

Dr. Ingber emphasized in a keynote presentation the importance of the tumor microenvironment and showed that local changes in extracellular matrix (ECM) remodeling and cell mechanics actively contribute to tumor initiation and progression. He also showed that breast cancer cell growth and spheroid architecture are normalized when mixed with normal embryonic mesenchyme or with the critical ECM component biglycan in either 3D Matrigel or in collagen I gels (1). This insight on the tumor microenvironment was applied in the context of human 'organs-on-chips': cell culture devices containing micrometer-sized chambers seeded with live

cells that then recapitulate the specialized multicellular architectures, tissue-tissue interfaces, physicochemical microenvironments, and vascular perfusion necessary to capture complex organ-level functions and human disease processes *in vitro* (2), (3). These chips permit real-time, high-resolution imaging of cellular and molecular processes within an organ-level context (*e.g.*, tumor-on-a-chip systems) that could complement or even bypass the use of animal models and potentially serve as new tools for more effective, low-cost drug screening.

### ***Vascularized Tissue Engineered Systems***

Dr. Gerecht highlighted the importance of incorporating vascular components into tissue engineered biomimetic systems since angiogenesis is vital for tumor growth and cancer cell migration. She stressed that the ECM promotes tumor angiogenesis and described the use of engineering approaches such as 3D ECM scaffolds, micropatterning, and microfluidic devices to investigate the role of the ECM in cancer progression (4).

Dr. George described an *in vitro* microfluidic perfusable vasculature network generated by combining human-derived endothelial cells and fibroblasts in fibrin gels (5). Using a similar approach, he also produced an *in vitro* prevascularized tumor model in which tumor cells co-cultured with endothelial cells and fibroblasts in fibrin gels form vascularized tumor spheroids (6). Individual tumor cells intravasating into blood vessels can be observed in this model. Future applications include studying the effect of intraluminal shear stress on endothelial cell-cell junction permeability during metastasis and the incorporation of prevascularized tumors into the microfluidic vascular network. These applications would facilitate the understanding of cancer

biology in the context of perfusion or may potentially be used for patient-specific drug development.

Dr. Soker introduced a method of decellularizing liver tissue from various animal models while preserving the integrity of the native ECM scaffold, tissue vasculature, and key bioactive molecules (7). Infusion of the decellularized scaffold with hepatocyte stem cells and human umbilical vein endothelial cells resulted in liver-like tissue containing a bile duct, hepatocytes, and vascular structures (8). Additionally, smaller-scale, liver-derived ECM discs mimicking native tissue can be seeded with metastatic tumor cells in a bioreactor, providing a useful high-throughput screening model for examining how environmental perturbations and physical forces affect liver metastasis or assessing anti-cancer drug efficacy.

Dr. Chen presented his work on engineering capillary blood vessel networks with precisely defined geometries *in vitro*. Photolithographic patterning or 3D printing technologies are used to generate microfluidic channels in 3D collagen. Endothelial cells seeded into these channels form a confluent endothelium, and application of angiogenic cocktails induces sprouting of new perfusable microvessels (9), (10), (11). Dr. Chen showed that perfusing these systems supports interstitial parenchymal cells and cell trafficking between the vascular and interstitial space. He emphasized that constructing the simplest *in vitro* biomimetic systems while faithfully capturing complex biological processes could contribute novel insights into how interactions between cancers and vasculature affect cancer growth and metastasis.

### ***Biomimetic Systems for Probing the Tumor Physical Microenvironment***

Dr. Kumar discussed how the tumor microenvironment is comparable in importance to cell-autonomous properties for understanding tumor progression. He described how physical features of the microenvironment regulate glioblastoma progression in terms of cell-intrinsic factors (*e.g.*, actomyosin contractility (12)), cell-extrinsic factors (*e.g.*, ECM rigidity, ECM porosity, chemical gradients (13)), and the cross-talk between these factors (14).

Dr. Guelcher introduced the clinical problem of metastasis-associated bone disease and showed that, using 2D polyurethane (PUR) films with tunable mechanical properties, a stiff microenvironment such as bone promotes osteolytic gene expression (15). He found similar results using a 3D perfused bioreactor incorporating a PUR matrix with tunable pore size and elasticity. Dr. Guelcher proposed the development of both systemic and local anti-cancer drug delivery from bone grafts to target pathways involved in osteolysis-promoting crosstalk between tumor and bone. Proposed next generation scaffolds would be bone-templated to assess matrix resorption and to compare bone morphology and pathology.

Dr. Wong presented the use of polydimethylsiloxane (PDMS) stencils to pattern cells and monitor migration of metastatic melanoma cells co-cultured with epithelium or fibroblasts from different organs (*i.e.*, microenvironmental niches). Cell migration data showed that in contrast to the random migration pattern of tumor cells alone, tumor cells in co-culture migrated directionally towards the niche cells. Dr. Wong discussed future studies to incorporate anatomical features of specific organs *in vitro* using PDMS stencils and to identify factors associated with organ tropism of metastatic melanoma.

Dr. Harley discussed the development of biomaterial “rheostats” to study glioblastoma cell migration and invasion by selective modification of the extrinsic microenvironment (*e.g.*, microstructural/mechanical or compositional changes, cross-linked or soluble biomolecules, heterotypic cell-cell interactions). He reported a methacrylated gelatin hydrogel platform to assess malignancy of glioblastoma cells under varying levels of epidermal growth factor receptor activity (16). To recapitulate spatio-temporal heterogeneity in gliomas, he developed a microfluidic device with optically transparent gradient hydrogels containing overlapping patterns of cell and matrix components with the ability to retrieve sub-regions of the construct for downstream analysis (17). Dr. Harley presented future plans to use such chip-based platforms to study glioma-immune cell interactions.

### ***Correlation of Molecular Phenotypes and Tissue Function with Biomimetic Systems***

Dr. Shuler reviewed the concept of “body-on-a-chip” and discussed the importance of utilizing “physiologically based pharmacokinetic models” to integrate multiple organ representations into a system with common, defined cell culture media. This is important for accurately mimicking organ metabolism and function when assessing a tissue construct’s response to genetic manipulation or exposure to cancer therapeutics (18).

Dr. Griffith in a keynote presentation highlighted the power of systems biology to integrate information across length scales (*e.g.*, molecular, cellular, extracellular) by using data from patient samples, animal models, and *in vitro* biomimetic systems to computationally predict



cellular response to therapy. She showed that multiplex molecular profiling of inflammatory cytokines from endometriosis patient samples predicted many inflammatory networks driving endometrial cell invasion, a process similar to cancer metastasis (19), (20). Ultimately, this approach could be utilized to develop predictive therapies targeting multiple activation pathways simultaneously. She also described systems approaches to analyze how variations in hormonal, nutrient, and inflammatory status within a human 3D liver bioreactor differentially influence growth and chemotherapeutic responses of dormant vs. actively metastatic triple-negative breast cancer cells (21).

Dr. Vunjak-Novakovic described an *in vitro* model of Ewing's sarcoma (ES) within an engineered bone environment where perfused, native decellularized bone scaffolds were seeded with human mesenchymal stem cells (hMSCs) that differentiated into osteoblasts and deposited bone matrix. Microaggregates of ES cells were introduced into the engineered human bone. High-throughput gene expression analysis suggested that the engineered bone microenvironment resulted in re-expression of the original tumor phenotype and of native ES genes that are silenced in 2D culture (22). In another biomimetic system, she described hMSCs cultured in a collagen hydrogel with osteosarcoma cells. The hMSCs remodel the hydrogel, facilitating osteosarcoma cell migration and invasion.

Dr. Ludwig showed that ES cells grown in 3D electrospun poly( $\epsilon$ -caprolactone) (PCL) biomimetic scaffolds displayed growth kinetics, protein expression, and chemotherapeutic drug response more similar to *in vivo* xenograft tumor models than to ES cells grown in 2D culture (23). Bioreactor perfusion of the electrospun PCL scaffold mimicked fluid mechanical forces in

bone and enhanced nutrient supply, resulting in more uniform distribution of ES cells throughout the scaffold and better long-term cell survival. He proposed that this biomimetic system could be a powerful tool for modeling metastasis and identifying drugs targeting the tumor niche.

Dr. Kuo described an air-liquid interface for culturing long-term intestinal organoids that remain viable for up to one year (24). Combinations of up to four genetic alterations converted primary colon organoids to adenocarcinomas that maintained tumorigenicity when transplanted into mice (25). Dr. Kuo proposed using such organoids for oncogene discovery by applying systems approaches to derive prioritized lists of potentially oncogenic alterations from The Cancer Genome Atlas datasets, genetically modifying the organoids according to the lists, and functionally validating their tumorigenicity *in vitro* and *in vivo*. He also proposed an organoid-based chemotherapeutic screening assay where results could be correlated with patient outcome to propose recommendations for treatment.

Dr. Ewald discussed his research on understanding the cell behavioral basis of breast cancer metastasis. He showed that primary human breast tumor organoids cultured in 3D reconstituted basement membrane were relatively indolent, whereas organoids derived from the same patient and cultured in stromal collagen I were highly invasive (26). Basal epithelial markers, including Keratin 14 (K14), were specifically expressed in the invasive leader cells of both mouse- and human-derived tumor organoids. While the mechanism is unclear, the K14<sup>+</sup> leader cell phenotype is inducible where bulk luminal K14<sup>-</sup> cells can become K14<sup>+</sup> and lead invasion. Importantly, knocking down K14 gene expression in tumor organoids inhibits collective invasion both in 3D culture and *in vivo* (27).

## Future Directions

Tissue engineered biomimetic systems hold great promise for providing an attractive alternative or complement to the current cancer research experimental models, namely, 2D cell culture (which can lack important parameters of the microenvironment) and *in vivo* mouse model systems (which can be costly and may not reflect what occurs in human cancers). Summarized below are challenges and opportunities to advance biomimetics for cancer research.

Improved understanding of the physical microenvironment. With biomimetic systems there is an advantage of being able to control one or more specific parameters of the physical microenvironment. Because tissue architecture affects gene expression profiles and cellular function, biomimetic systems need to be developed to study the interplay between genotype, phenotype, and cell extrinsic factors including stiffness, topology, pH, and oxygen tension. Biomimetic systems could also be extremely valuable if they can accurately recapitulate the physical and chemical properties of local microenvironments encountered by cancer cells at distinct steps of metastasis. For example, though not discussed at the workshop, extravasation of circulating tumor cells (CTCs) from the bloodstream into metastatic sites can be recreated by developing endothelial networks within microfluidic devices to assess how CTCs interact with endothelial cells to surpass the endothelial barrier and establish metastatic growth at secondary sites under physiologic flow and shear stress conditions (28). Recreating the physical and chemical parameters of a tissue-specific host microenvironment with biomimetic technologies

could also provide insight into how the ECM and heterotypic cell-cell interactions influence CTC colonization and the tumor dormancy switch in the metastatic niche (29), (30).

Development of biomimetic systems and predictive computational models to understand spatio-temporal dynamics in cancer. To better understand tumor progression and recapitulate cancer complexity, it will be important to fabricate *in vitro* systems that correlate physiologic, macroscopic tissue architecture with micro- or nano-scale molecular markers. Such biomimetic systems representing the dynamics of physiological responses will increase insights into how tumor cells integrate mechanical, electrical, chemical, and structural signals within the microenvironment over time at multiple length scales. Multi-scale computational modeling approaches are also desired to better integrate multiple data sets and information about tumor heterogeneity and the microenvironment in order to predict tumor dynamics and treatment response.

Accelerated drug discovery. Biomimetic systems could serve as a tool for (1) screening new drugs or combinations of drugs, (2) optimizing dosing strategies, and (3) developing personalized therapy by utilizing organoids or patient-derived tumor xenografts (PDX) for downstream analysis. Eventually, biomimetic systems could provide a widespread platform for developing high-throughput assays that are more cost-effective and efficient than *in vivo* models, yet more physiologically relevant than 2D cell culture systems. To this end, the NIH has developed the Microphysiological Systems (MPS) Program, a partnership with the National Center for Advancing Translational Sciences, the Defense Advanced Research Projects Agency, and the Food and Drug Administration to accelerate research in drug toxicity and efficacy (31).

As the MPS program does not solely address cancer therapy, more concerted efforts could be made in this area, perhaps by coordinating efforts with other programs that support the use of conditionally reprogrammed cells, organoids, and PDX tumors to understand the mechanisms of therapeutic response and resistance. Development of personalized medicine could be further enhanced by utilizing human stem/progenitor cells or induced pluripotent stem cells that can be programmed to recreate specific 3D organ systems mimicking metastatic sites (32), (33). Incorporation of tumor cells derived from PDX tumors or human organoids into these stem cell-derived platforms could thus provide a patient-specific means for therapeutic screening that takes into account the tumor microenvironment.

Creating physiologically relevant biomimetic systems. It will be important to develop biomimetic tumor models that contain the essential components necessary for accurately recapitulating *in vivo* conditions without being overly complex and therefore prone to difficult data interpretation. Although *in vivo* models are valuable, it is not possible to control and monitor individual parameters with the precision offered by biomimetic systems. Development of novel biomaterials to accurately mimic mechanical and topological properties of the tumor microenvironment is important, as is the development of advanced technologies to measure subcellular and supracellular mechanical properties. Incorporation of immune system components is needed to gain a more comprehensive understanding of how immune cells in the microenvironment interact with tumors to affect cancer progression (*e.g.*, assessing the effect of co-culturing tumor-specific T cells with tumor cells). Lastly, it will be important to use patient-derived tumor and stromal cells to overcome the genotypic and phenotypic drift that occurs over

time in immortalized cell lines, but challenges remain in the collection and propagation of primary cells and their maintenance variability in different experimental settings.

Validation of biomimetic systems. The development of embedded molecular or chemical probes to track microenvironmental changes and individual cell behavior in 3D scaffolds is important for validation of biomimetic systems. An advantage would be the generation of corresponding real-time, cell-based functional readouts and multiplex analysis, thereby allowing the comparison of tumor formation and anti-cancer drug efficacy in different models such as 3D matrices, traditional cell culture assays, *in vivo* xenograft models, and *in vivo* histological sections. A challenge of system validation is the appropriate selection of a “gold standard” for comparison, which depends on the biological question under investigation.

Improved reproducibility. Development of different biomimetic tissue engineered systems to understand cancer complexity requires the establishment of guidelines for calibration and reproducibility. Criteria could include the incorporation of essential calibrated biosensing modalities to measure O<sub>2</sub> and pH, development of biomaterials and scaffolds calibrated to *in vivo* tumor conditions, DNA profiling to ensure authenticity of cell lines, the generation of a human cancer bank for supplying human tumor cells, and standardized cell culture methods.

Transdisciplinary teams. Investigators with diverse backgrounds should collaborate with one another to provide novel perspectives for applying biomimetic tissue engineered systems to cancer research. Required expertise may include tissue and microsystems engineering, biomaterials, cancer biology, clinical oncology, and pharmacology. Engineers, physicists,

materials scientists, and computational experts working very closely together with those who have a deep understanding of cancer pathophysiology will be able to define the most pertinent, basic and applied cancer biology research questions and develop applicable engineering tools to test these focused and well-defined questions.

## **Summary**

The research presented at this workshop showcased important advances in the application of various biomimetic tissue engineered systems to probe questions in cancer research. Recent work highlighted the use of 3D synthetic scaffolds, bioreactors, decellularized tissue, and *ex vivo* tissue cultures to better understand the chemical and mechanical interactions between tumors and the microenvironment and to decipher the dynamics of genetic and molecular changes that occur during cancer progression. Of particular note was the potential clinical application of biomimetic systems as more efficient and economical tools for therapeutic screening, the importance of utilizing computational models for creating integrated biomimetic tumor systems, and the need to fabricate reproducible, physiologically relevant biomimetic platforms that adequately capture the essential components of a physiologic system without being overly complex. Overall, participants from diverse research backgrounds and expertise emphasized the importance of transdisciplinary collaborations for best defining the pertinent clinical and biological cancer research questions that can be addressed with the appropriate biomimetic tissue engineered technologies.

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